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Spectrum of Mutations in the *OCRL1* Gene in the Lowe Oculocerebrorenal Syndrome

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Summary

The oculocerebrorenal syndrome of Lowe (OCRL) is a multisystem disorder characterized by congenital cataracts, mental retardation, and renal Fanconi syndrome. The *OCRL1* gene, which, when mutated, is responsible for OCRL, encodes a 105-kD Golgi protein with phosphatidylinositol (4,5)bisphosphate (PtdIn[4,5]P_2) 5-phosphatase activity. We have examined the *OCRL1* gene in 12 independent patients with OCRL and have found 11 different mutations. Six were nonsense mutations, and one a deletion of one or two nucleotides that leads to frameshift and premature termination. In one, a 1.2-kb genomic deletion of exon 14 was identified. In four others, missense mutations or the deletion of a single codon were found to involve amino acid residues known to be highly conserved among proteins with PtdIns(4,5)P_2 5-phosphatase activity. All patients had markedly reduced PtdIns(4,5)P_2 5-phosphatase activity in their fibroblasts, whereas the ocrl1 protein was detectable by immunoblotting in some patients with either missense mutations or a codon deletion but was not detectable in those with premature termination mutations. These results confirm and extend our previous observation that the OCRL phenotype results from loss of function of the ocrl1 protein and that mutations are generally heterogeneous. Missense mutations that abolish enzyme activity but not expression of the protein will be useful for studying structure-function relationships in PtdIns(4,5)P_2 5-phosphatases.

Introduction

Lowe oculocerebrorenal syndrome (OCRL; McKusick 309000) is an X-linked disorder characterized by congenital cataracts, mental retardation, and Fanconi syndrome of the proximal renal tubules (Lowe et al. 1952). The *OCRL1* gene has been cloned (Attree et al. 1992) and encodes ocrl1, the protein product of the *OCRL1* gene, a 105-kD phosphatidylinositol(4,5) bisphosphate (PtdIns[4,5]P_2) 5-phosphatase that is deficient in OCRL patients (Suchy et al. 1995; Zhang et al. 1995).

We previously reported mutations in three unrelated OCRL patients, two of whom had the same nonsense mutation whereas the third demonstrated an exon-skipping mutation leading to frameshift and premature termination (Leahey et al. 1993). However, the majority of OCRL patients have no detectable *OCRL1* mRNA (Attree et al. 1992), and therefore reverse transcriptase–PCR (RT-PCR) generally is not suitable for screening for all *OCRL1* mutations. To search for mutations in genomic DNA, the *OCRL1* intron/exon structure was mapped, 24 exons were identified, and primers flanking each translated exon were designed to amplify each exon from genomic DNA (Nussbaum et al. 1997). Nine more mutations were identified, eight by analysis of exons amplified from genomic DNA and one by Southern blot analysis. We also have identified three additional mutations by RT-PCR and sequencing in patients in whom *OCRL1* mRNA is detectable by northern blotting.

Material and Methods

Patient Samples

Cultured lymphoblastoid or fibroblast cell lines were obtained, with informed consent of parents or guardians, from male patients carrying a clinical diagnosis of OCRL, based on the phenotypic triad of congenital cataracts, Fanconi syndrome of the proximal renal tubules, and mental retardation.

PCR Methods

The 23 coding exons of *OCRL1* and their flanking intronic sequences were amplified from patient genomic DNA obtained from lymphoblastoid or fibroblast cells as follows. Reactions were performed with 50–100 ng of *Bam*HI-digested genomic DNA, 0.5 μM each of forward and reverse primers specific for each exon, 1.25 U

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of *Taq* polymerase, 200 μmol of dNTP, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.001% gelatin in 20 μl. A 98°C soak for 30 min was followed by 35 cycles of 94°C for 1 min, an annealing step for 1 min, and 72°C for 1 min (Gene Amp PCR system 9600; Perkin Elmer). The annealing temperature for the first 10 cycles was decreased sequentially by 1°C and was held constant for the remaining 25 cycles. Primer sequences are available through the World Wide Web on the Springer-Verlag website for Human Genetics (http://www.springer.de).

SSCP Analysis

Each exon was amplified by use of the above PCR conditions, with one primer end-labeled with ^{32}P-γ-ATP. PCR products were denatured in loading buffer (95% formamide, 10 mM EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue) at 98°C for 5 min and immediately were cooled on ice and separated on a nondenaturing Hydrolink MDE gel (AT Biochem) at 4°C by electrophoresis at 45 W.

Sequencing

Exons of each patient were amplified as described above. A DNA cycle sequencing kit (Promega) used the primer end-labeled with ^{32}P-γ-ATP. The sequencing procedure consisted of 30 cycles of 95°C for 20 s, annealing at the appropriate temperature for 20 s, and 70°C extension for 40 s. Annealing temperatures varied from 45°C to 55°C. The PCR products then were separated on 6% acrylamide gel with constant current of 75 Ws for 2–3 h. Bands were visualized on x-ray film (Kodak) at −70°C.

Southern Blot Analysis

Genomic DNAs from patients were digested with *Bam*HI, *Eco*RI, or *Xba*I and were separated on a 0.9% gel. DNA was transferred to nylon membrane (Schleicher & Schuell) in 10 × SSC. The membrane was hybridized with a cDNA probe that contained exons 12–15. The filter was washed once at 65°C for 10 min in 2 × SSC and 0.1% SDS and twice in 0.2 × SSC and 0.1% SDS for 10 min.

RT-PCR

Reverse-transcription reactions and sequencing of RT-PCR products were performed as described elsewhere (Leahey et al. 1993).

PtdIns(4,5)P_2 5-Phosphatase Assay

Cell extracts were prepared from patient fibroblasts by freeze-thawing. Activity was assayed as described elsewhere (Suchy et al. 1995). Western blotting also was performed as described elsewhere (Olivos-Glander et al. 1995).

Results

The diagnosis of OCRL was confirmed biochemically in all 10 of the 12 patients for whom fibroblasts were available for enzyme assay. Enzyme assay in these 10 patients revealed activities in the range of 0.05–1.02 nmol/min/mg (table 1). Enzyme activities in this range are all <2 SDs from the mean ± SD of the activity for OCRL patients (0.55 ± 0.29 nmol/min/mg) and are >6 SDs below the mean ± SD for the activity of normal control fibroblasts (6.41 ± 0.81 nmol/min/mg).

Screening with SSCP of RT-PCR products or PCR products of genomic DNA revealed aberrant migration of fragments in 11 patients. Exons with aberrant SSCP bands were sequenced. In one patient, an exon could not be amplified, and genomic DNA was examined by Southern blot analysis for a large genomic deletion or insertion. With this approach, 11 different mutations were found in 12 patients, as shown in table 1.

Four patients carried 1- or 2-bp deletions leading to frameshift and premature termination ⩽12 codons downstream. Three additional patients were found to have nonsense mutations that were predicted to truncate the ocrl1 protein prematurely during translation. Western blot analysis of all six of the seven patients for whom fibroblasts were available revealed no detectable ocrl1 protein.

Exon 14 could not be amplified in the cell line from patient XL82-02, whereas exons 13 and 15 were amplified readily. Southern blot analysis with a 402-bp cDNA containing exon 12 through part of exon 15 identified an ~1.2-kb genomic deletion including exon 14. No protein was detectable by immunoblotting.

Three missense mutations and one in-frame codon deletion were identified. Patient XL59-01 had a 3-bp deletion in exon 12, causing an in-frame codon deletion of either T350 or T351. Patient XL78-07 showed an aberrant SSCP band derived from exon 13 after SSCP. Sequencing identified an A→G missense mutation at base 1529 of the gene, changing a highly conserved aspartic acid at position 434 to a glycine. Patient cell line PHL255 had an A→G substitution at nucleotide 1748, which changes histidine 507 to arginine in exon 15. These missense mutations and the single codon deletion abolished PtdIns(4,5)P_2 5-phosphatase activity, whereas the ocrl1 protein, although reduced, still was detectable by western blotting. Patient LS-15 had a T→C substitution at nucleotide 1565 in exon 14, changing a conserved phenylalanine 446 to serine. Fibroblasts were not available for enzyme assay or western blotting. A schematic diagram summarizing the mutations in this report, as well as two nonsense mutations reported elsewhere (Leahey et al. 1993), is shown as figure 1.

Discussion

Twelve mutations were identified in the *OCRL1* gene in OCRL patients. Three of them were found with RT-

Table 1

Location, Nucleotide Change, Amino Acid Change, Enzyme Activity, and Protein Expression in 12 Patients with OCRL

Patient	Exon	Mutation Type	Nucleotide Change (Base Number[a])	Predicted Effect on Translation	Enzyme Activity[b,c]	Protein[c,d]
XL58-01	10	1-bp deletion	1051delG	Frameshift and stop	.34	0
LS24-01	15	1-bp deletion	1695, 1696, or 1697delA	Frameshift and stop	.53	0
LS23-01	21	2-bp deletion	2535–2536, 2536–2537, or 2537–2538delGT	Frameshift and stop	1.02	0
LS38-01	21	2-bp deletion	2535–2536, 2536–2537, or 2537–2538delGT	Frameshift and stop	.61	0
XL54-02	10	Nonsense	1060C→T	Q278X	NA	NA
XL49-22	12	Nonsense	1339C→T	Q371X	.79	0
LS36-01	18	Nonsense	2164C→T	R646X	.24	0
XL82-02	14	Exon deletion	genomic deletion	. . .	.60	0
XL78-07	13	Missense	1529 A→G	D434G	.10	1+
LS15-01	14	Missense	1564T→C	F446S	NA	NA
PHL255	15	Missense	1748 A→G	H507R	.25	2+
XL59-01	12	3-bp deletion	1276–1278 or 1279–1281delACC	del T350 or T351	.05	1+

[a] According to GenBank entry U57627.

[b] Data are means of duplicate assays. Mean ± SD PtdIns(4,5)P_2 5-phosphatase activity for normal fibroblasts is 6.41 ± 0.81 nmol/min/mg (n = 7); and that for OCRL fibroblasts is 0.55 ± 0.29 nmol/min/mg, (n = 23).

[c] NA = not available.

[d] Scale is from 0, for no protein detectable, to 4+, for normal protein levels.

PCR followed by sequencing, and the rest were found by amplification of genomic DNA from each exon, followed by SSCP and sequencing.

Eleven independent, distinct mutations were identified in the *OCRL1* gene in these 12 OCRL patients. In seven, a nonsense mutation or a deletion of one or two nucleotides led to frame shift and premature termination. In one, a 1.2-kb genomic deletion of exon 14 was identified. In the other four, missense mutations or the deletion of a single codon was found. Eleven of the 12 mutations in this study are different, as expected in an X-linked genetic lethal disorder in which new mutation is likely to contribute a significant number of mutant alleles (Haldane 1935). Surprisingly, the same mutation,

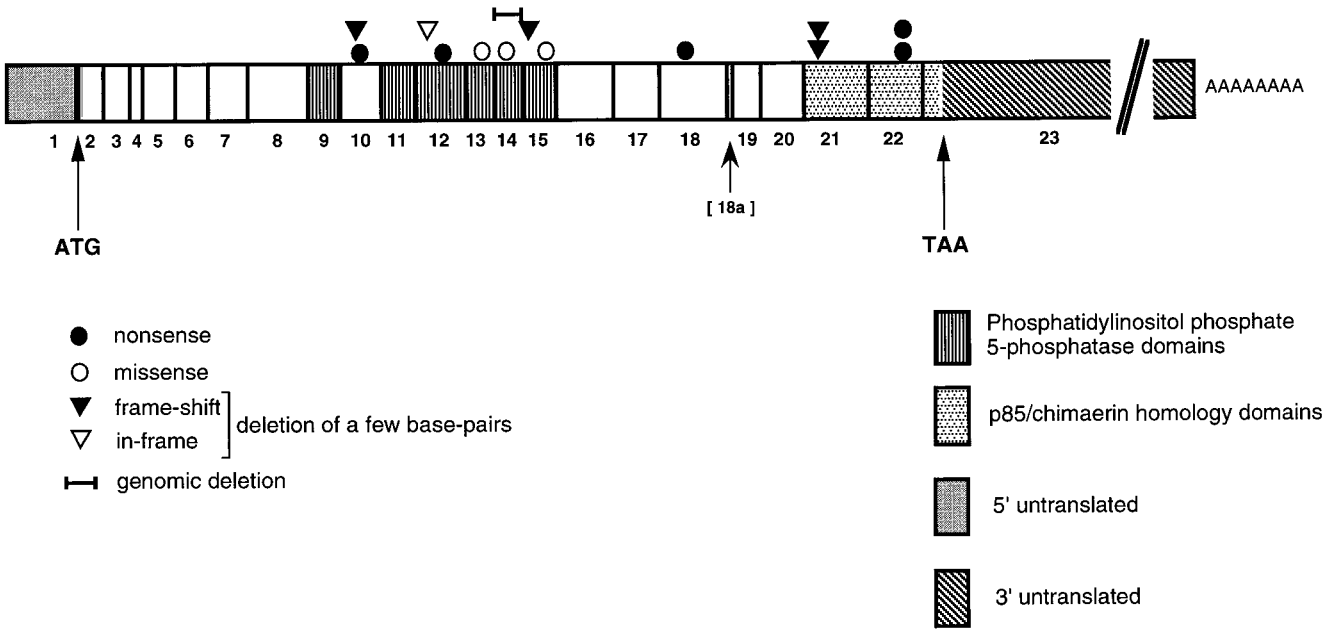

Figure 1 Schematic diagram of *OCRL1* cDNA depicting the location of the various mutations found in the gene. Exons are numbered 1–23, with an alternatively spliced exon shown as 18a. Except for the 3′ untranslated portion of the cDNA, exons are drawn to scale (Nussbaum et al. 1997).

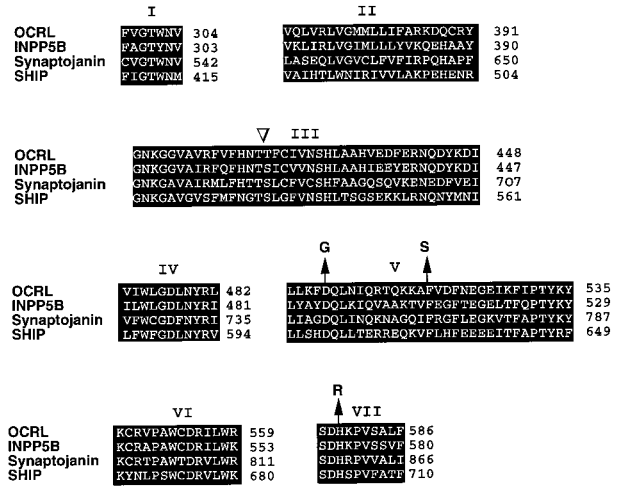

Figure 2 Seven blocks of highly conserved amino acid sequence shared by four PtdIns(4,5)P_2 5-phosphatases. Numbers indicate the amino acid residue for each protein and are derived from their GenBank entries (*OCRL1,* U57627; INPP5B, M74161; synaptojanin, U45479; and SHIP, U39203). Arrows indicate locations of highly conserved amino acid residues that are either altered to another amino acid or deleted in the *OCRL1* gene of OCRL patients. Protein alignments were carried out by the MACAW Multiple Alignment Construction and Analysis Workbench using the segment pair overlap method under a BLOSUM62 scoring matrix (Schuler et al. 1991; Henikoff and Henikoff 1993). Statistical significance was assessed under the null hypothesis of random alignment under a search space defined as the lengths of the actual sequences.

a 2-bp deletion in exon 21, did recur in two unrelated patients. Recurrence of a nonsense mutation due to a C→T transition also was seen elsewhere in two other unrelated patients with OCRL (Leahey et al. 1993).

Besides ocrl1, seven highly conserved protein domains have been described (Erneux et al. 1995; Jefferson et al. 1995; R. L. Nussbaum, M. Sheahan, A. Dutra, and M. Budarf, unpublished data) among four mammalian proteins with known PtdIns(4,5)P_2 5-phosphatase or phosphotidylinositol (3,4,5)P_3 5-phosphatase activity (Suchy et al. 1995; Zhang et al. 1995): inpp5b, originally described as an inositol 5-phosphatase in platelets (Ross et al. 1991; Matzaris et al. 1994; Jefferson et al. 1995); synaptojanin, a protein involved in synaptic-vesicle cycling (McPherson et al. 1996); and ship, an *SH*2 domain–binding *i*nositol *p*hosphatase that participates in signal transduction (Damen et al. 1996). In figure 2, domain I is in exon 9, domain II in exon 11, domain III in exon 12, domain IV in exon 13, domain V in exons 13 and 14, and domains VI and VII in exon 15. As shown in figure 2, the three missense mutations and the one codon-deletion mutation are located in domains III, V, and VII of the seven highly conserved motifs of proteins with PtdIns(4,5)P_2 5-phosphatase activity (Olivos-Glander et al. 1995). In three of these patients for whom fibroblasts were available, enzyme activity was reduced to levels comparable to that seen in patients with completely null mutations, whereas the ocrl1 protein still was readily detectable by western blotting. Jefferson and Majerus (1996) used site-directed mutagenesis to target regions, in inpp5b and ship, containing domains IV and VI, as shown in figure 2. They identified certain conserved amino acids that were required for substrate binding and hydrolysis of phosphate from inositol polyphosphate and phosphatidylinositol polyphosphate substrates. These mutations, therefore, are in domains that are distinct from the domains reported here as having mutations in OCRL patients. The naturally occurring mutations reported here, combined with the mutations engineered by Jefferson and Majerus, provide additional biological support for the functional importance of five of the seven highly conserved domains in PtdIns(4,5)P_2 5-phosphatase proteins.

The PtdIns(4,5)P_2 5-phosphatase assay proves to be an accurate tool for diagnosing OCRL. Carrier detection, however, is not feasible by enzyme assay, because, in contrast to autosomal genes that cause metabolic disease, *OCRL1* is X linked and not expressed from the inactive X chromosome (Hodgson et al. 1986; Mueller et al. 1991). Random X inactivation is likely to lead to a broad range of enzymatic activity, depending on the fraction of cells in the sample that have inactivated the X chromosome carrying the normal *OCRL1* gene. Direct mutation detection does provide a dependable carrier test in those families in which the mutation is known. In this sample of 12 unrelated probands, five of the mutations changed restriction sites, and one caused a shift in the size of a fragment seen by Southern blot, thereby providing a simple and convenient way to screen the family members for carrier status at the OCRL locus. Other allele-specific tests are being developed for carrier testing in the remaining families.

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